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Effect of Cold Plasma treatment on fungi inactivation and germination of maize grains (*Zea mays* L.)

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Abstract:
Cold plasma (CP) technology is one of the most promising technique. This study investigated the efficiency of CP treatment to inactivate fungus - contaminated maize grains and their effect on germination. The grains were exposed to CP at two different potencies for 10, 20 and 30 min. Antifungal effect was observed only for the grains treated with CP submitted to the higher potency and longer period of exposure. In relation to the germination of maize grains, the PC was not efficient. CP has been shown to be a safe decontamination procedure that can be applied sustainably to maize during storage (whose germination is not necessary).
Key Word*:Maize;Cold Plasma; Germination;Inactivation; Fungi.*

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I. Introduction

Plasma is a partially ionized neutral gas, also known as the 4th state of matter that is highly energized containing ions, electrons and neutral reactive species (radicals, excited atoms and molecules). It also includes ultraviolet (UV) radiation with enough energy to break covalent bonds and/or initiate several reactions (Moisan et al., 2002; Niemira et al., 2002; Niemira, 2012; Sen at al., 2012; Bourke at al., 2018).

As far as CP food applications are concerned, due to its non-thermal characteristics, it has been utilized (as a heat treatment substitute) for vegetables (fruits / grains) and animal origin proteins, such as grain cooking time reduction, different types starch modifications , as well as to keep shelf life longer.It is also applied for enzyme and microorganisms inactivation (Niemira, 2012; Schnabel at al., 2014; Hertwig et al., 2014). There are three main mechanisms by which CP inactivates microorganisms i.e., through (a) chemical interaction of radicals, reactive species or cell membrane loaded species; (b) damage to membranes and internal cellular components by UV radiation and (c) UV DNA strand breaks generated during a recombination of plasma species. The mode of action may be more significant in one product than another and the greater sanitizing effectiveness results from CP with multiple antimicrobial mechanisms (Moisan et al., 2002; Bolshkov et al., 2004; Gallagher et al., 2007).

Cereals comprise of a quite diversity of crops and supplies, where more than 50% is aimed for human consumption (Poutanen et al., 2014). CP technology, one of the most promising non-thermal techniques utilized in the cereal industries (Mir et al., 2016). Maize (*Zea mays* L.) is one of the world's major cereals, with a production of 1,1 billion tons annually. The United States, China and Brazil are the largest producers, accounting for 70% of world production (Conab, 2019). Maize grains have their quality changed when they are infected by fungi, which are potential mycotoxins producers. That contamination causes damages to human and animal health due to its toxic activity (Kumar et al., 2008; Scussel et al., 2018). The field and storage fungi that contaminate maize are mainly species from genus *Fusarium*, *Aspergillus* and *Penicillium* (Scussel et al., 2018).

In other to control / prevent fungal spoilage / contamination, new technologies that, do not generate toxic compounds / modify the nutritional properties or palatability of the grain and its products, have been developed (Eman, 2010). Several green methods have been studied, including gases (ozone, carbon dioxide, nitrogen), plant extracts (jucá – *Caesalpinaferrea*L., guarana – *Paulliniacupana* K., andiroba (*Carapa guianensis* Aubl.) and CP (Scussel et al., 2010; Martins, 2014; Savi et al., 2014; Silva et al., 2018b; Runtzel et al., 2018a,b).

Therefore, this study investigated the efficiency of CP technology application to inactivate fungi naturally contaminated maize grains at different conditions and its effect on germination.

II. Material And Methods

2.1 Material

(a) Sample:Maize grains (total: 1 Kg), naturally contaminated (total load-$0.4x10^4$ CFU/g), kindly provided by the Integrated Company of Agricultural Development of Santa Catarina – CIDASC, Brazil.

(b) Culturemediumandother material: Potato dextrose agar (PDA), Kasvi (Santa Catarina, Brazil), chloramphenicol, Vetec (Rio de Janeiro, Brazil), Petri dishes/plates (60mm diameter), Kasvi (Santa Catarina, Brazil); Drigalski loop, Prolab (São Paulo, Brazil).

(c) Equipment:Stomacher, Marconi (Piracicaba, SP); colonycounter, Phoenix (São Paulo, Brazil); autoclave, Phoenix (Araraquara, SP, Brazil); bacteriologicaloven, Fanem (São Paulo, SP, Brazil); dryingoven, Quimis (Diadema, SP, Brazil); analyticalscale (0.01-210 g), Ohaus (Parsippany, NJ, USA); laminar flowchamber, Veco (Campinas, SP, Brazil). CP system – a reactor (glass chamber – size: 11.5x10.5 cm, with silicone cap and oxygen gas input) with dielectric barrier plasma jet. One high voltage (± 17 kV) alternating current source 30 mA was employed to generate 240 and 360 W plasma. A high voltage source was connected to a VARIAC ATV-215-MP transformer (220-240V; 60 Hz; 6.3 A; 1-1.5 kVA) which is used to control the voltage supplied. The generation of CP occurs in the reactor, by electric current – alternating – induces the breakdown of gas molecules. The process generates electrons accelerated by an electric field, forming the CP.

2.2 Methods

(a) Mycota from maize grains (total fungi count): The mycological tests were carried out from portion (25 g) of maize sample aseptically weighed as follows: The portion were transferred to polyethylene bag and added peptone water (0.1%) followed by homogenization (2 min at stomacher); then, a volume (100 µl) of each diluted sample (10^{-1}; 10^{-2} and 10^{-3}) was inoculated (n=3) on PDA surface containing chloramphenicol (100 mg/L) in a flow laminar cabinet and incubated at 25±1°C for 7 days (Silva et al., 2013; APHA, 2015). Their total count was read using the colony count and recorded as colonies forming units (CFU/g). Only the same dilution plates that had 15 to 150 colonies were counted.

(b) Preparation of maize Groups: Grains were arranged on previously prepared petri dishes with PDA – one grain / dish (Figure 1). They were divided into two main groups: Control group (GC - without CP treated) and treated group (GT_1 and GT_2- CP treated at 240 and 360 W power, respectively) for 10, 20 and 30 min of CP exposure. The analyses were performed in triplicates for GC and GTs. ***(c) Treatment with CP:*** The GT plates were placed into the reactor (glass chamber), followed by sealing (silicone cover) and electrode positioning, then applied the power parameters of 240 and 360 W, at intensity of 8 kV and different exposure times (10, 20 and 30 min). After CP application on the grains, both groups (GTs and GCs) were evaluated for ***(1) fungal growth inhibition:*** Plates were incubated at 25ºC for 5 days and the possible antifungal CP effects were monitored through fungi growth evaluation (mycelia formation / reduction) *versus* time of exposure for inactivation efficiency (compared to GC) and ***(2) post-treatment grains characteristics:*** The germination capacity was evaluated by monitoring their embryo tissues changes through the development of the coleoptile and primary root (radicle) and compared to the control behavior.

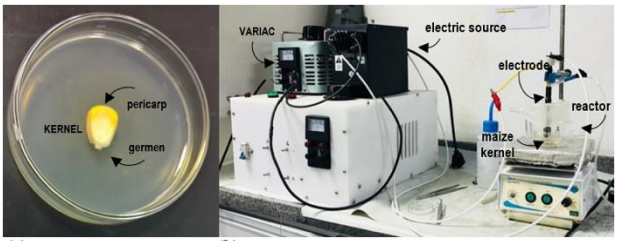


Figure 1. Cold plasma (CP) experiment: (a) maize grain (*Zea mays* L.) arranged on PDA Petri dish center for further CP treatment and (b) CP equipment with maize grain inside reactor.

III. Result

The data obtained from maize (naturally contaminated) treated with CP at different power parameters (240 and 360 W) and exposure times (10, 20 and 30 min), showed its efficiency, with variations, depending the conditions applied. Table 1 shows the CP (a) antifungal and (b) germination effect on grains, respectively.

3.1 CP antifungal effect

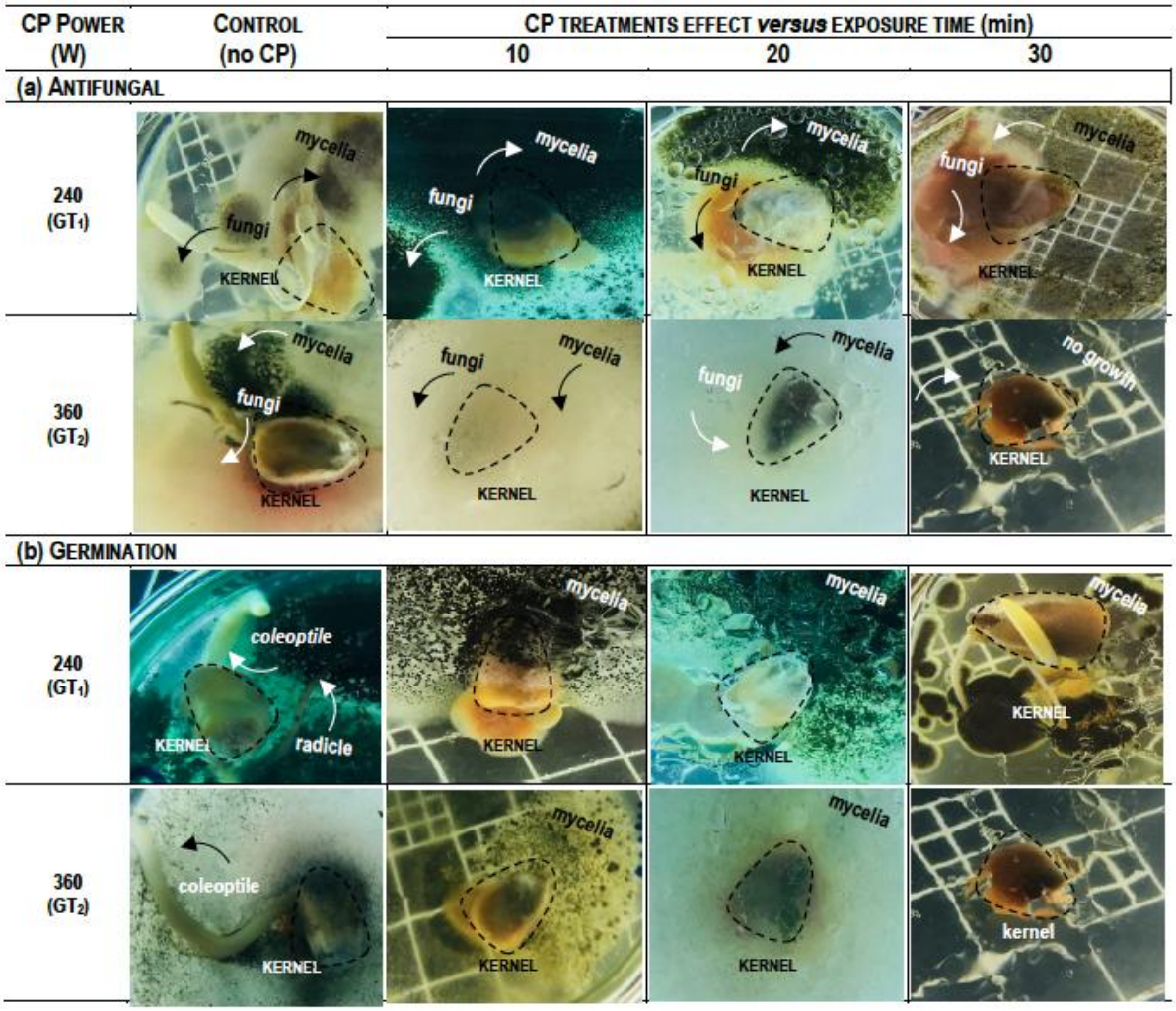
Regarding the antifungal effect, after treating grains with CP, it was observed on the 5th day of incubation that only the GT_2 group (power: 360 W, exposure: 30 min) had total fungal growth inhibition (33.33% inactivation) (Table 1). The other treatment conditions, regardless the exposure times (10, 20 and 30 min), allowed fungi growth (100%)**.**

Some studies have reported the CP treatment effectiveness against fungi in grains and pulses. Lee et al. (2016) and Devi et al. (2017) observed that a reduction in the microbiota (bacterium and fungi) in rice (*Oryza sativa* L.) and peanuts (*Arachis hypogaea*L.), as well as Pizá (2018b), on infected soybean (*Glycine max* L.) seeds. In addition, Balestrasse (2016)achieved 100% germination and vigor recovery. In wheat (*Triticumaestivum*L.), it was reported that *Fusarium nivale*was more sensitive to CP treatment, reaching a total growth inhibition (100%) in only 2 min.

However, for *Aspergillus flavus*, Zahoranová et al. (2015) reported that total inhibition only was achieved by applying twice the time for *Fusarium* i.e., 4 min. The efficacy of CP against filamentous fungi on wheat seeds surface decreased in the following order: *F. nivale> F. culmorum>A. flavus >A. clavatus*. According to Pizá (2018a), active plasma agents (such as ions, free radicalsand UV radiation, among others) react with biomolecules and destroy them, making the pathogenic microorganisms and toxins harmless, by weakening their cell membrane, resulting in their inactivation**.**

The data obtained in the current study, corroborated partially with those authors findings. It is important to emphasize that the study materials utilized (apparatus / samples type) were not the same, as the CP equipment (each equipment has a different operation and may vary the results from one equipment to another) making it somewhat difficult to compare. In addition, its fungi load may be higher and / or the fungi genera present more resistant to the CP technique in parameters (the voltages and times) applied. Regarding, fungi load, it also plays a role in the effectiveness of treatment (especially whether the infection attacks the surface or interior of the maize grain (germ tissue - blue eyes).

Table 1. Evaluation of cold plasma (CP) effect* on naturally contaminated maize grains (*Zea mays* L.) for antifungal and germination by applying different power intensities and exposure times [*at Day 7th incubation]



3.2 Maize grains CP effect on germination

Germination is a grain particularity that has been reported being related to the CP technology and seed quality. Despite that, there are some controversies related to it (Stolairi et al., 2006; Randeniya& De Groot,

2015; Bormashenko et al., 2015; Matias et al. 2017). In the current study, germination by CP application did not show to be that effective. Only, the GT at the lower power applied (240 W) and time (at 30 min) kept the germination capacity(33.3%).

Some studies have shown that plasma is able to favor not only seed germination, but also the development of plants (Randeniya& De Groot, 2015; Bormashenko et al., 2015). According to Silva et al. (2018a), the treatment of *Hybanthus calceolaria* seeds with CP for 1 min favored its germination. Jiafeng et al. (2014) observed that treatments at 80 W power significantly improved the potential and germination rate in wheat when compared to Control. However, Matias et al. (2017) obtained unsatisfactory results for the germination of melon seeds.

In seeds, germination is of extreme importance, as it is the initiation process of the plant growth. In the case of the current study, the grains are aimed (for consumption to (a) produce flour for food processing or (b) sell as whole grain in the retail market. Therefore, its non-germination is an advantage (especially during their storage) for the industry, considering that the consumer has no interest in buying germinated grains.

It is worth noting that each researcher uses a different CP equipment. That factor ends up influencing the final result, since, variations can occur from one equipment to another. Also referring to the gas used, power, electrodes types, reactor geometry, energy applied, among other possible oscillations (Silva et al., 2018b). In the case of maize, the non-germination may have been induced by the long exposure time and/or high power, damaging the structure responsible for germination, or by factors related to equipment differences. Despite that, if the aim of the CP treatment is to prevent / control fungi growth in stored grains (for flour production mills), lack of germination will not be a problem, but a solution. In addition, maize grain may be more sensitive to treatment than other grains interfering in its germination capacity.

IV. Conclusion

The CP treatment showed a potential to reduce fungal load on maize grains being the best condition: 360 W power for 30 min exposure. Regarding germinating, it affected grain behavior, as it inhibited its performance. More studies on the CP effects on maize need to be carried out utilizing other conditions, including sensorial evaluated.

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